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(54) Title: BIOLOGICAL PREPARATIONS AND THEIR USE

(57) Abstract

Immunotherapeutic agents prepared from Mycobacterium vaccae are useful in the treatment of mycobacterial disease, especially tuberculosis or leprosy, in particular as an adjunct to chemotherapy.

BIOLOGICAL PREPARATIONS AND THEIR USE

This invention relates to immunotherapeutic agents useful in the immunotherapy of mycobacterial disease, especially tuberculosis and leprosy.

The eradication of mycobacterial diseases such as tuberculosis and leprosy by effective treatment is still a primary objective particularly in disease endemic areas such as third world countries of Asia, Africa and South East Asia. Modern drug treatment of these diseases consists of chemotherapy with, for example, rifampicin and isoniazid in the case of tuberculosis and clofazimine and sulphones in the case of leprosy.

Chemotherapy, though effective in killing rapidly metabolising bacilli, is very slow to eliminate "persisters", and this necessitates continuation of treatment for 9 months to a year in the case of tuberculosis, and 5 years or more in the case of leprosy. 'Persisters' are metabolically inactive microorganisms which can survive long exposure to a drug, only becoming susceptible when they start to multiply.

We have now found that the mycobacterium, M. vaccae, is especially effective for the immunotherapy of mycobacterial disease, especially tuberculosis and leprosy. Experiments have shown that suspensions

H_3BO_3	5.25 g
NaCl	6.19 g
Tween	0.0005%
Distilled Water	to 1 litre

The preferred strain of M. vaccae is one denoted R877R isolated from mud samples from the Lango district of Central Uganda (J.L. Stanford and R.C. Paul, Ann. Soc. belge Med, trop. 1973, 53, 141-389). The strain is a stable rough variant and belongs to the avium sub-species. It can be identified as belonging to M. vaccae by biochemical and antigenic criteria (R. Bonicke, S.E. Jahasz., Zentr albl. Bakteriol. Parasitenkd. Infection skr. Hyg. Abt. 1, Orig., 1964, 192, 133). M. vaccae is believed to be closely similar antigenically to M. leprae (J.L. Stanford et al, British Journal of Experimental Pathology, 1975, 56, 579).

The strain denoted R877R has been deposited at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

For the preparation of the immunotherapeutic agent, the microorganism M. vaccae may be grown on a suitable solid medium. A modified Sauton's liquid medium is preferred (S.V. Boyden and E. Sorkin., J. Immunol, 1955, 75, 15) solidified with agar.

administered as an adjunct to chemotherapy, and normally 1 to 3 months after starting effective chemotherapy, e.g. with one of the chemotherapeutic agents mentioned above. Thus its effect is designed to be maximal after the majority of bacilli in the lesions, i.e. the metabolically active bacilli, have been killed and the load of bacterial antigenic material has begun to decline.

The invention therefore includes within its scope a method of treating mycobacterial disease, e.g. tuberculosis or leprosy, which comprises administering to a subject suffering therefrom antigenic material derived from Mycobacterium vaccae in an amount sufficient to provoke an immune response effective against metabolically inactive cells of mycobacteria.

The immunotherapeutic agent is believed to have two modes of action. It presents the "protective" common mycobacterial antigens to advantage and contains immune suppressor determinants active in regulating disadvantageous immune mechanisms (P.M. Nye et al, Leprosy Review, 1983, 54, 9). As a result of its action, "persister" bacilli are recognised by the immune system by their content of common mycobacterial antigens and effective immune mechanisms are directed against them, in the absence of the tissue necrotic form of immunity usually present in mycobacterial disease (G.A.W. Rook & J.L. Stanford, Parasite

microorganisms are then harvested and weighed and suspended in diluent (1 part Tween 80 in 300 parts saline) to give 100 mg of microorganisms/ml of diluent. The suspension is then further diluted with saline to give a suspension containing 10 mg of microorganisms/ml of diluent and dispensed into 5 ml multidose vials. The vials containing the live microorganism are then subjected to radiation from ^{60}Cobalt at a dose of 2.5 megarads to kill the microrganisms and give the immunotherapeutic agent of the invention, which may (if desired) be further diluted for use.

This immunotherapeutic agent may be administered by intradermal injection in the manner already described.

10. Dead cells of Mycobacterium vaccae for use in therapy in the treatment of mycobacterial disease.

11. Killed cells of Mycobacterium vaccae NCTC 11659 for use in therapy in the treatment of tuberculosis or leprosy.

12. An immunotherapeutic agent according to any one of claims 1 to 8 for use in therapy in the treatment of tuberculosis or leprosy.

13. Antigenic material from Mycobacterium vaccae for use in therapy in the treatment of mycobacterial disease as an adjunct to chemotherapy.

14. Method of treating mycobacterial disease which comprises administering to a subject suffering therefrom antigenic material derived from Mycobacterium vaccae in an amount sufficient to provoke an immune response effective against metabolically inactive cells of mycobacteria.

15. Method according to claim 14 in which the mycobacterial disease is tuberculosis or leprosy and the mycobacteria are Mycobacterium tuberculosis or M. leprae.

16. Method according to claim 14 in which the antigen material comprises dead cells of M. vaccae.

17. Method according to claim 14 in which the antigenic material comprises cells of M. vaccae NCTC11659 which have been killed by irradiation.

18. Method according to claim 14 in which

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00064

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 39/04

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Infection and Immunity, volume 20, no. 2, May 1978, Washington (US) F.M. Collins et al.: "Immune response to persistent Mycobacterial infection in mice", see pages 430-438, especially page 437, lines 30-54	1-13
X	Biological Abstracts, volume 69, no. 1, 1980, Philadelphia (US) S.R. Watson et al.: "Delayed hypersensitivity responses in mice and guinea pigs to Mycobacterium leprae, Mycobacterium vaccae and Mycobacterium nonchromogenicum cytoplasmic proteins", see page 306, abstract 2847, Infect.Immun. 25(1)229-236,1979	1-13
X	Biological Abstacts, volume 78, no. 3, 1984, Philadelphia (US) F.M. Collins et al.: "Fernandez and Mitsuda reactivity in guinea pigs sensitized with heat-killed Mycobacterium leprae: persistence and	

* Special categories of cited documents:
"A" document defining a general state of the art which is not considered to be of particular relevance
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"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

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International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	G.L.M. Kruithof

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